# Sporothrix (Sporotrichum) schenckii in a Nursery Barn Containing Sphagnum

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URING A SEARCH for Blastomyces dermatitidis and other fungi pathogenic to man in their natural habitats in Wisconsin, it seemed desirable to try to isolate all pathogenic fungi, including B. dermatitidis, not only from sites frequented by persons or dogs with blastomycosis but also from the premises of persons with other systemic and subcutaneous fungus diseases. There is evidence that two species of human pathogenic fungi may exist in the same niche, although each species may have its own peculiar range of habitats in nature. Histoplasma capsulatum and Cryptococcus neoformans, for example, have both been isolated a number of times from chicken houses (1-3). While the number of isolations of C. neoformans has often, but not always, been found to be relatively small in comparison with the number of isolations of *H. capsulatum*, the number isolated depends on the geographic area of study (3). A recent demonstration by Walter and Yee (4) that high concentrations of extracts of chicken dung tend to inhibit the growth of C. neoformans may explain the low prevalence of this

Dr. McDonough is a professor and Miss Lewis is a research associate in the department of biology, Marquette University, Milwaukee, Wis. Dr. Meister is an assistant clinical professor in the department of dermatology, Marquette Medical School, and an attending physician at the Milwaukee County Hospital in Milwaukee and the Veterans' Administration Hospital in Wood, Wis. fungus in poultry houses. This possible tendency may also explain why, in Kentucky (2), this pathogen was found only in chicken houses with low concentrations of chicken manure.

A case of sporotrichosis in a man working in a large nursery near Milwaukee, Wis., afforded us an opportunity to study another species of fungi, besides B. dermatitidis, that can cause subcutaneous or systemic disease in human beings. The nursery where the man worked had consistently used a large amount of sphagnum moss, and several patients in Wisconsin with blastomycosis had reported contacts with this kind of moss. Furthermore, sphagnum had been incriminated by several investigators as a reservoir of infection for Sporothrix (Sporotrichum) schenckii (5-8). Recovery of S. schenckii during the early stages of our investigation led us to try to eradicate the pathogen from the barn where it was found.

## Case of Sporotrichosis

The patient with sporotrichosis was 38 years old. The physician who first treated him thought that he had a cellulitis on his left wrist. The man stated that in the spring of 1964 he had stuck himself with a thorn on the back of his left wrist. The physician ordered warm compresses, elevation of the arm, and one 250 mg. tetracycline capsule four times a day. The man showed no improvement, however, after 5 days of treatment and was referred to Meister for consultation.

Meister first saw the patient on June 18, 1964. An examination at that time revealed a granulo-matous lesion on the dorsum of the patient's left wrist, measuring approximately 1½ by 1½ cm., and also, two small papules above the original granulomatous lesion. These lesions appeared to be in the lymphatic chain.

A biopsy specimen of the granulomatous tissues, as well as smears and cultures, were studied for fungus cells, "tubercles," and bacteria. The biopsy revealed focal acanthosis and hyperkeratosis of the squamous epithelium. Microscopic examination also revealed an area of ulceration with a granulomatous reaction; the ulcerated area contained aggregates of neutrophiles, histiocytes, lymphocytes, and cellular debris. Giant cells were scant and not prominent. Routine H. and E. (hematoxylin and eosin) stains revealed cellular and nuclear debris, but no demonstrable fungi. PAS (periodic-acid Schiff) stains, including Gridley's modification, were used, as well as the gram stain and the Gomori-Grocott silver method. The silver stains showed cigar-shaped cells, each with a prominent wall. These cells measured approximately 2 by 1  $\mu$  and showed irregular focal septation. Bacteriological examination of the blood agar medium confirmed the presence of cigar-shaped bodies, and S. schenckii was positively identified on Dubois oleic agar. This result was later confirmed by the State Laboratory of Hygiene, University of Wisconsin Medical Center.

On June 30, 1964, Meister prescribed a saturated solution of potassium iodide for the patient. The initial dose was 5 drops of potassium iodide in water three times a day before meals; this dosage was increased daily by 3 drops until the patient was taking 40 drops three times a day. By October 15, 1964, all the affected areas appeared to be healed, but medication was continued for 2 more months. The patient tolerated the therapy well, except for development of a mild acneiform eruption on his back and, to a lesser extent, on his face. The iodide reaction subsided after the treatment was stopped.

## **Epidemiologic Background**

The patient's only recollection of having been injured in the weeks immediately preceding the onset of the disease was when he had been stuck with a thorn or splinter while handling barberry, Russian-olive, hawthorn, and rose plants in the nursery barn. His disease developed at a time when the shrubs of the nursery were in storage.

The nursery had used the barn where he was injured for many years solely for storing shrubs from the period before the ground froze solid in the fall until the plants were sold again in the spring. The barn provided about 6,500 square feet of floor space in three adjacent rooms, all on the same floor. The cement floor was broken through in two places to provide drain holes, about 8 inches in diameter, which dead-ended in the soil. Steel beams supported the ceiling. Most of the walls were of masonry.

In storing the shrubs, the practice was to cover the roots with moist sphagnum after most of the soil had been removed from the roots and to stack the plants in rows on the floor of the barn. The sphagnum was kept moist during storage. By late spring, the plants had been sold, and the barn was usually empty until October except for wood benches and the like, left over from the spring sales, and small pieces of sphagnum, left over from sweeping. The sphagnum to be used during the fall and winter was not brought into the barn until early fall.

## Methods in Field and Laboratory

The samples that we used in assaying for pathogenic fungi were collected from the barn with sterile instruments and were placed in sterile, leak-proof 8-ounce plastic bags. Each sample contained at least five portions collected at random in a designated area in the building. Samples taken from large floor areas on which there was very little soil, sphagnum, and so forth consisted of 20 to 25 portions obtained by scraping the floor with a sterile spoon. We took samples of all kinds of solid organic materials present in the barn, the number of samples of each kind collected depending on the relative frequency of the material at the time of collection. In each of the last seven collections, we tried to obtain samples from the entire barn. Samples were collected 10 different times.

We recognized at the start of our investigation that the purchase and use of many new bales of sphagnum each fall might serve to reintroduce S. schenckii into the nursery barn (5, 8). For this reason, we tried in the fall of 1965 and of 1966 to sample at random and to assay a large number of the bales for the fungus before they were used. With sterile tongs, at least five portions of sphagnum were taken from each bale and combined into one sample. We took the moss from near the center and from deep in each quarter of the bale.

Soil, wood, sphagnum, and so forth were assayed for pathogenic fungi by direct and indirect procedures (9, 10). We inoculated each mouse used in the indirect procedure intravenously, as well as intraperitoneally, with suspensions of the materials collected. The suspensions were also plated on brain-heart-infusion dextrose agar with antibiotics at 37° C. Small samples of sphagnum were also embedded in a yeast extract agar containing antibiotics (11) and in Sabouraud dextrose agar containing 100 units of penicillin, 0.1 mg. of streptomycin, and 1 mg. of cycloheximide per milliliter (12).

Since the men in the nursery objected to the use of formaldehyde in the barn, it was not feasible to use this disinfectant, which has been found to be effective against *H. capsulatum* in field soil (13). For all experiments except some preliminary work, we decided to use a 2 percent

Table 1. Materials collected at nursery barn and samples found positive for Sporothrix (Sporotrichum) schenckii, 1964-66

Materials sampled	Number of samples collected	of samples
In barn		
Sphagnum and soil in the two		
drain holes	17	6
Sphagnum and soil from floor		-
(including shallow cavities)	75	17
Sphagnum on floor	24	3
Sphagnum around roots and so		
forth	38	6
Unused sphagnum from 52		
bales	<b>52</b>	1
Used sphagnum on screening,		
cement blocks, empty water	11	5
tanks, and laths	$\frac{11}{2}$	ő
Tar paper	10	ŏ
Dry wood	10	ĭ
wei wood	1	
Outside of barn		
Pile of used sphagnum	20	0
Total	250	39

solution of Amphyl (a commercial product with a mild odor, which contains potassium ricinoleate, o-phenylphenol, p-tert-amylphenol, and alcohol, and is produced by Lehn & Fink Corporation). This compound, one of the widely used hospital and laboratory bactericides and fungicides, is similar to other phenolic compounds that have previously been shown, under controlled conditions, to be effective in killing pathogenic fungi on various surfaces (14, 15). The nurserymen did not object to use of the Amphyl solution in the storage barn even when workers were present.

#### Results

The shrubs in the nursery barn had all been sold or put back in the field by the time that the disease was diagnosed, the fungus from the patient had been grown in the laboratory, and we had been notified of the results. By then, the barn had been completely cleaned out except for the wooden furniture used to support plants; soil and sphagnum also still filled the drain holes in the floor. While the lower layers of the mixture in the holes were moist, the inside of the barn and its contents were very dry.

We made our first collection of samples on September 1, 1964, from the empty barn. From that date through December 28, 1966, we collected 250 samples from the barn or the immediate area. The composition of the samples and the number that were found positive for S. schenckii are shown in tables 1 and 2.

On December 22, 1964, with plants in the barn, we collected samples from places in it where our previous collection had shown the pathogen to be present and also from other likely places. Samples from unused bales of sphagnum, from a pile of used sphagnum discarded behind the barn, and from two shallow cavities in the floor did not yield S. schenckii. The fungus was isolated, however, from the mixture of soil and moss in the two deep drain holes and in two of the shallow cavities in the concrete. It was also isolated from sphagnum which had been moistened after being placed over piles of shrubs.

The mixture of sphagnum and soil apparently served as a reservoir for the fungus. The only material in the barn which had remained moist each year during the growing season when the barn was not being used was this mixture. Furthermore, the cavities were located near the door. Thus, as shrubs were brought into the barn, the infested mixture of sphagnum and soil was scattered throughout the barn and more sphagnum and soil were deposited in the holes.

We decided to try to destroy the reservoir of the fungus. Therefore, on December 22, 1964, after taking samples, we soaked the debris in the two drain holes with Amphyl and sprayed the disinfectant on the surface of the floor about each hole (table 2). The workers did not find the mild odor of the Amphyl objectionable. We encouraged them to use heavy gloves when working with barberry, roses, and so forth.

On April 19, 1965, we visited the nursery again. Plants were being sold in the barn; it was still crowded with them. Shrubs were piled over all the cavities in the floor except a large drain hole nearest to the door. A sample was taken from this opening in the floor. We also took samples from the floor within a 3-foot radius of the hole, from the sphagnum and soil between the drain hole and the door, from sphagnum around roots of plants piled around the hole but well off the floor, from sphagnum and soil along a path leading into the barn between the piles of plants, from sphagnum around the roots of plants along the path but

well off the floor, and from sphagnum left over from previous years which was piled outside of the barn.

Since S. schenckii had been isolated in December 1964 from sphagnum which had been placed on a pile of plants, we expected that the fungus would be widely scattered in the barn by April 1965. Such was the case. On April 19, 1965, the pathogen was isolated from the moss and dirt mixture near the drain, from the floor on the path to the door and on the path extending inward away from the hole, and from sphagnum around the roots of plants. We did not recover any fungus, however, from the debris in the drain holes. Possibly the mixture in the drains had retained enough of the Amphyl so that S. schenckii would not grow in it. Nor was any fungus recovered from the old sphagnum dump outside the barn.

On July 24, 1965, we soaked all the drain holes and cavities in the floor which contained soil and sphagnum with Amphyl and sprayed the floor with this disinfectant in a 3-foot circle around each hole or cavity. These steps were taken on the assumption that these areas were the chief sources of contamination each year by S. schenckii.

In August and September of 1965, following this soaking, we took 30 composite samples from

Table 2. Isolations of Sporothrix (Sporotrichum) schenckii from samples of materials collected in nursery barn before and after disinfection

Dates	Condition of barn	Samples collected 1	Samples positive	
	Condition of parti		Number	Percent
Dec. 22, 1964	Empty	19 10	7	37 70
July 24, 1965	ril 19, 1965do	7	4	57
ng. 24, 1965	17 13	3	12 0	
July 23, 1966 Sept. 10, 1966	Plants in barn	34 24 17	4 7 6	12 29 35
Sept. 24, 1966	and all floors and walls sprayed. Empty Plants in barn	5 32	0	0
Total		1 178	38	21

<sup>&</sup>lt;sup>1</sup> 20 samples from a pile of used sphagnum which had been discarded outside of the barn over the years and 52 samples taken from new bales of this moss at

delivery are not included. These samples are referred to in the "Results" and the "Discussion."

the empty nursery barn, and only three yielded S. schenckii. One positive sample came from a soil and sphagnum mixture; the other two were comprised of relatively pure sphagnum collected from some of the screening in the barn and from an empty water container; this container was distant from the drain hole and the cavities in the floor that contained soil and sphagnum. One of 24 new bales of sphagnum which, through an error, were unfortunately put into the barn in the fall of 1965 without first being assayed for fungi, was shown to harbor S. schenckii. It seems likely that this one bale was contaminated by being in contact with the floor as the sphagnum was being placed in the barn. Thirty-three bales from the same shipment had been sent to another nursery in the area, and samples from these 33 bales revealed no S. schenckii.

The decrease in the proportion of positive samples—from 70 percent on December 22, 1964, when the barn contained plants, to 12 percent in the empty barn on August 24, 1965, after the soaking and spraying—suggests that soaking the drain holes and floor cavities was resulting in the elimination of the pathogen. Apparently the sphagnum could act as a reservoir to harbor the pathogen, allowing it to survive in the barn from one year to the next. An increase in the percent of positive samples in the collections made in the summer and early fall (July 28 and September 10, 1966) strengthened this view. Therefore on September 17, 1966, the nursery men soaked the drain holes and floor cavities with Amphyl and sprayed the entire floor and all walls with it. No S. schenckii was isolated from any of the 37 samples taken on September 24 and December 28, 1966.

## Characteristics of Isolates

S. schenckii was the only fungus pathogenic to human beings that we isolated during our investigation. The S. schenckii strains were isolated only by the indirect mouse technique. The isolates were all pathogenic for mice, as was shown by the growth of the organism in the liver, lung, and spleen and by the formation of abscesses in the lower part of the peritoneal cavity.

The morphological and physiological characteristics of the S. schenckii isolates from the

nursery barn were typical of the species and were generally identical with the traits of the isolate which had been obtained from the patient with sporotrichosis. At 25° C., the conidia were borne in rosette patterns on delicate sterigmata at the tips of conidiophores and in solitary fashion on delicate sterigmata along a hypha. When grown at 37° C. on brain-heart-infusion dextrose agar, conversion to the yeast form occurred. Typical blastospores were formed that were commonly cigar-shaped, often oval, and infrequently round. Complete conversion to the yeast form was typically slower than that shown by the human isolate, several transfers being necessary in some cases. The dimensions of the conidia and the blastospores fell within the ranges given by previous investigators (16-19). The isolates from the barn were brown or black when grown for the first time at room temperature, a characteristic typical of primary isolates from human beings and animals. This trait was sometimes lost after frequent subculturing. All isolates grew well on media containing 1,000 µg. of cycloheximide (Acti-dione) per milliliter.

#### Discussion

It is not known when or how the nursery barn originally became contaminated with S. schenckii. The two drain holes in the floor that contained soil and sphagnum apparently never dried out completely during our investigations and had no doubt harbored the pathogen for years. Wood in the barn may also have acted as a reservoir of contamination. Early work by other investigators (20) has indicated that the spores of S. schenckii can remain alive in a dried condition for 4 years. Growth of the fungus and the production of new spores, however, requires ample moisture and a favorable temperature. The moist board which had been standing in the drain hole in the barn yielded S. schenckii, while 10 samples taken from dry boards did not. Undoubtedly other cases of sporotrichosis would have occurred among the nursery workers if it had not been the practice to remove all plants, sweep the floor, and allow the barn to dry out each year.

We do not know why plants that were set out in the fields in the spring and brought back into the barn in the fall did not recontaminate the barn each year. We found no evidence, however, that they did. Furthermore, one need not postulate the entrance of the fungus into the barn each year through the air or by some vector in order to account for the continued presence of S. schenckii. No doubt the pathogen remained in the building from one year to the next.

There was little evidence that the new sphagnum purchased each year acted as a vector. One new bale (of 85 bales assayed) gave evidence of harboring S. schenckii. This bale, however, could have been contaminated after it was put in the barn and before it was examined for the presence of the fungus. The likelihood that the new bale was contaminated after it was brought into the barn is heightened by the fact that 33 other bales from the same shipment, which were placed in another barn at another nursery, were not found to be harboring S. schenckii. Also, no S. schenckii was found in the samples from 28 new bales which had been purchased in the fall of 1966 but not placed in the barn until after it was disinfected.

We do not claim that the results of our investigation show that the collecting and storage facilities of the primary suppliers of sphagnum are free from S. schenckii. Whether or not this is the case has yet to be proved. If these facilities are found to harbor the pathogen, an opportunity for further testing of the control measures we have outlined will be at hand.

The question as to whether or not S. schenckii lives in the bogs is a separate problem that is being pursued by other investigators. At present, this question would seem to be equivocal. The careful work of Christensen and Whittingham (21) resulted in the isolation of "Sporotrichum schenckii?" through dilution plating from two of five open bogs which had a substratum of sphagnum and sedge peat, from one of five spruce and tamarack swamps with sphagnum in the ground layer, and from two of five white cedar and balsam fir swamps containing peatforming wood and leaf debris (swamps which were overgrown with a variety of Bryales mosses but where little or no sphagnum was present). As these investigators have indicated, the identity of this fungus is questionable. Christensen allowed us to examine the original isolate from the bog. We agree with her that the morphological characteristics of this isolate would allow its inclusion in the species S.

schenckii as defined by several authors (16-19) and that it is similar to the avirulent strains of Howard and Orr (17). We had no difficulty, however, in distinguishing the bog isolate microscopically from S. schneckii isolates which were undoubtedly pathogenic. Moreover, we were unable to infect mice with the isolate from the bog or to convert it to the yeast form. We were also unable to induce it to grow at 37° C. or at 25° C. on cycloheximide agar (Sabouraud dextrose agar plus 0.5 mg. of Acti-dione per milliliter). All of the isolates of zoopathogenic S. schenckii that we have examined have shown both of these capabilities. Some investigators (22) consider pathogenicity to be an essential characteristic of S. schenckii.

During our investigation, we observed that used sphagnum taken from the barn and dumped into a pile outside failed to yield S. schenckii a year later (20 samples assayed). The cause of this failure is uncertain. Possibly, however, this phenomenon may be the result of a failure of the pathogen to live out of doors through a Wisconsin winter. S. schenckii strains that are undoubtedly pathogenic have apparently not been isolated frequently, if at all, from exposed situations in the northern States. On the other hand, several investigators have reported isolations from geographic regions in the Americas with milder climates, such as California (17), Brazil (23), and Uruguay (22). But before we conclude that pathogenic S. schenckii cannot survive severe winters, definitive experiments should be done, especially in view of the recent report of experimental work by Ahearn and Kaplan (24) which indicates that some strains of this species may be adapted to grow at low temperatures.

Even if all S. schenckii could be eliminated from the sphagnum used in horticulture or sphagnum were to be no longer used, the danger from sporotrichosis would probably not be greatly reduced unless a program of eradication such as we have outlined were followed. Certainly, storage of plants in humid places at a temperature favorable for root preservation favors the growth of S. schenckii. This fungus may be able to grow wherever horticultural plants are stored even if sphagnum is not present. The world's greatest reservoir of S. schenckii infection—more than 3,100 cases in

human beings before 1961—seems to be the gold mines of South Africa. where this fungus grows on the wooden timbers used as mine props (20). It has been clearly shown that S. schenckii will grow not only on wood, sphagnum, and soil, but also on many other materials such as straw (22, 25).

We do not claim to have eliminated S. schenckii from the barn we investigated. Certainly, however, the number of infectious particles in this building, as well as the chances for infection, were greatly reduced. We observed no injury to plants, nor was any such injury called to our attention, following the use of Amphyl. This disinfectant therefore seems to afford an effective and inexpensive method for the control of S. schenckii. Without doubt many other substances could be used effectively in controlling similar S. schenckii contaminations (14, 15). It has been shown that a number of substances which preserve wood also have helped to prevent S. schenckii from growing on support timbers in South African mines (20). If Amphyl should be found injurious to growing plants, another fungicide could be substituted. One of the organic fungicides recently tested for the elimination of S. schenckii from soil might be tried (26).

#### Summary

Sporothrix (Sporotrichum) schenckii was isolated repeatedly over a 3-year period from a nursery storage barn in which a man had presumably acquired sporotrichosis. The evidence indicated that S. schenckii had been carried from one year to the next in a mixture of sphagnum and soil in drain holes and cavities in the barn's cement floor, as well as occasionally on moist wood or used sphagnum.

Flooding the drain holes and cavities with a phenolic fungicide reduced the contamination. More complete control was obtained when the walls and ceiling of the empty barn were also sprayed with the disinfectant. Following disinfection, 37 samples taken at random from all over the floor and from piles of plants were negative for S. schenckii.

Failure to isolate S. schenckii from used sphagnum which was assayed a year after it was placed outside the barn indicates a need for studying the effect of wintering on this fungus.

Conclusive evidence that the pathogen was introduced into the barn in new bales of the moss was not obtained; nor was it demonstrated that S. schenckii was introduced from the surrounding fields.

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#### **Tearsheet Requests**

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# **Public Health Service Staff Appointments**

William E. Cox has been appointed special assistant for physical therapy affairs. He is the first physical therapist to be assigned to the immediate staff of the director of Community Health Service, the Health, Education, and Welfare unit responsible for the professional health aspects of the Medicare program.

In his new capacity, Mr. Cox will assist the director with physical therapy matters involving governmental programs both within and outside the Public Health Service and with nongovernmental physical therapy organizations and activities.

Mr. Cox will continue to serve as physical therapy consultant to the Division of Health Resources, Community Health Service. He has been a member of the Public Health Service Commissioned Corps for 16 years. He received his certificate in physical therapy from Baylor University Medical Center in Dallas, Tex., following his B.S. degree from Southern Methodist University. He also holds a master's degree in public health from the University of Michigan.

Edgar N. Duncan has been named Assistant to the Administrator of the Health Services and Mental Health Administration with special interest in equal employment opportunity. He will be deputy equal opportunity officer for the Office of the Administrator.

Mr. Duncan's first objective will be to conduct "a survey from which a talent data bank will be established for employees in the Office of the Administrator to enable these employees to move up the job ladder as far as their capabilities and talents will take them."

Prior to his appointment Mr. Duncan served as Acting Director of the Office of Program Planning and Evaluation, Indian Health Service. A member of the Public Health Service Commissioned Corps, he holds the rank of Pharmacist Director or Captain.

A native of Monessen, Pa., Mr. Duncan holds a B.S. degree in pharmacy from Duquesne University and a master's degree in hospital administration from the University of Pittsburgh.